

Claims:

1. A method for the generation of living tissue-like organization of cells, viz., macromass culture, including three-dimensional tissue-like constructs, free from the requirement of scaffold or extraneous matrix, comprising:

a culture system in which cells are seeded at a high density per unit area of a culture vessel in a range spanning a window around  $10^6$  cells per  $\text{cm}^2$  resulting in three-dimensional tissue-like formation or organization of cells, free from the requirement for any other agents that aid in tissue formation.; and

providing tissue like constructs made from mesodermal cells, and could be applicable to other cell types;

2. The method as claimed in claim 1, including using macromass culture comprising a culture system for tissue formation, which comprises:

generating three-dimensional tissue-like organization, macroscopic or microscopic, from cells by high-density cell seeding; and

bringing the cells together in close proximity in a certain favorable range of high densities of cells in three-dimensional space, that favors cohesive integration of cells into a three-dimensional tissue-like state, free of the requirement for any other agents that aid in tissue formation.

3. Tissue-like organizations of cells including macroscopic three-dimensional constructs according to claim 1 for use as tissue substitutes for implantation, for wound healing, as in vitro models for drug testing, and the like, made from fibroblastic cells of mesenchymal origin, including:

engineering a putative dermal equivalent made from dermal fibroblasts, putative substitute with bone-like properties made from osteogenic cells derived from adipose stromal cells, and putative cartilage substitute made from chondrocytes, but not necessarily limited to these cell types; and

generating tissue-like organizations of cells which can be made to assume different forms.

4. Tissue-like organizations of cells as claimed in claim 3, which are three-dimensional in nature and encompasses three-dimensional macroscopic tissue-like constructs.

5. Tissue-like organizations of cells as claimed in claim 3, which can be made to assume different forms, being generated for the purpose of achieving different properties or qualities, said different forms comprising:

three-dimensional macroscopic tissue-like constructs by themselves, wherein “macroscopic” means that the size of the tissue is at least such that it can be easily visually discerned by normal human vision, and the macroscopic tissue-like constructs are histologically competent; and

combining the three-dimensional tissue-like organization with different matrices, such as gels, sheets, membranes or sponges or with other scaffolds and the like; and

said tissue-like organization being in the form of microscopic three-dimensional structures.

6. Tissue-like organizations of cells as claimed in claim 3, including tissue-like organizations which can be generated wholly using cells alone and a culture medium.

7. Tissue-like organizations of cells as claimed in claim 3, wherein the tissue-like organization of cells can be generated without using any agents that aid in tissue-formation, comprising:

tissue-inducing chemicals such as ascorbic acid;

tissue-inducing growth factors; and

substratum with special properties; rotational culture; complex bioreactor; or extraneous scaffold or matrix or supports.

8. Tissue-like organizations of cells as claimed in claim 3, including tissue-like organizations which can be generated free of the requirement for extraneous extracellular matrix components.

9. Tissue-like organizations of cells as claimed in claim 3, generated from different cell types comprising:

dermal fibroblasts;

adipose stromal cells;

osteogenic cells derived from adipose stromal cells;

chondrocytes; and

osteoblasts.

10. Tissue-like organizations of cells produced by macromass culture as claimed in claim 3, for the formation of which a range of cell seeding densities exists that favors tissue formation and the exact range of tissue-forming high cell seeding densities are possible to be different for different cell types.

11. Tissue-like organizations of cells by macromass culture as claimed in claim 3, wherein the time of formation can vary for different cell types or different media conditions.

12. Tissue-like organizations of cells produced by macromass culture as claimed in claim 3, generated free of the requirement for specific media conditions, including:

serum-free media conditions; and

specific complex media compositions.

13. Tissue-like organizations of cells produced by macromass culture and the different forms thereof as claimed in claim 3, that are three-dimensional and that have flexibility with respect to dimensions comprising:

different three dimensions sizes;

larger or smaller tissue-like constructs by scaling up or down macromass culture; and

variable size or scale by changing the number of cells used to achieve a seeding density within a macromass favorable range of tissue-forming densities by scaling up or down the macromass culture.

14. Tissue-like organizations of cells as claimed in claim 3, having a flexibility with respect to culture media used, comprising:

formation of the tissue-like organization in the presence of different culture media, both, different growth and/or tissue-formation media; and

modulating the properties of the tissue-like organization by including components in the growth and/or tissue-formation medium, provided that addition of these components does not adversely affect tissue formation, or by changing the medium, provided that this change in medium does not adversely affect tissue formation.

15. Tissue-like organizations of cells by macromass culture as claimed in claim 3, wherein the tissue substitutes are achieved on different compatible growth surfaces or scaffolds.

16. Tissue-like organization as claimed in claim 3, including the preparation of the tissue-like constructs made in culture vessels of any shape, with a flat or curved base.

17. Tissue-like organizations of cells as claimed in claim 3, which can be made to assume different forms, and different forms being generated for the purpose of achieving different properties or qualities, comprising:

three-dimensional macroscopic tissue-like constructs having a size that can be easily visually discerned by normal human vision;

the macroscopic tissue-like constructs being histologically competent;

combining the three-dimensional tissue-like organization with different matrices, such as gels, sheets, membranes or sponges or with other scaffolds; and

the tissue-like organization of cells being in the form of microscopic three-dimensional structures.

18. A method for the generation of tissue-like organization of cells including fabrication of three-dimensional tissue-like constructs free of the aid of scaffold comprising:

employing high cell-seeding-density culture to generate tissue-like organization of cells free of the requirement for employing specific agents that aid in tissue formation and scaffolds;

providing tissue-like constructs made from mesodermal cells, but not necessarily limited to these cell types; and

constructing the tissue-like organization of cells to produce different tissue engineered products by generating tissue-like organization of cells and formation of living, cellular putative tissue substitutes.

19. The method as claimed in claim 18, including using high cell seeding density per unit area or space of culture vessel free of the requirement for other agents to form the tissue-like organization of cells and to provide macroscopic tissue-like constructs.

20. The method as claimed in claim 18, including formation of tissue-like organization using macromass culture by seeding the cells at a high cell density per unit area or space of culture vessel.

21. The method as claimed in claim 20, wherein the macromass culture comprises a culture system for tissue formation, comprising:

seeding cells at a high density per unit area or space of the culture vessel in a range spanning a window around  $10^6$  cells per  $\text{cm}^2$  and free of the requirement for other agents that aid in tissue formation; and

the macromass culture further comprising:

generating tissue-like organization, macroscopic or microscopic, from cells by high-density cell seeding, bringing cells together in close proximity in a certain favorable range of high densities of cells in three-dimensional space, free of the requirement for any other agents that aid in tissue formation;

achieving the macromass range of favorable high cell seeding densities by settling the cells together within the three-dimensional space occupied by the cells at the base of the culture vessel such that they come into a state of close proximity with one another that triggers or signals them into a tissue formation mode by which they become cohesively integrated; and

achieving the macromass range of cell seeding density in a vessel with a flat or curved base whereby using a culture vessel of at least about 0.75 cm in diameter for macromass culture results in the formation of macroscopic tissue-like

constructs, and macroscopic defines a tissue size that can be easily visually discerned by the normal human vision.

22. Tissue-like constructs for implantation in a human or mammalian body, made in accordance with a method for generating macroscopic tissue-like constructs and whole tissue-like organization of cells free of the requirement for any specific agents to induce organization and can be scaled up to generate macroscopic tissue-like constructs free of the requirement for scaffolding material and specific agents/complex media formulations, solely by high cell-seeding-density culture, viz., macromass culture from cells comprising those of mesenchymal origin, wherein the tissue-like organization of cells and putative tissue equivalents made, are made from cells of mesenchymal origin including an engineered putative dermal equivalent made from dermal fibroblasts, putative substitute with bone-like properties made from adipose stromal cells-derived osteogenic cells or from osteoblasts and putative substitute for cartilage repair made from chondrocytes.

23. The tissue-like constructs as claimed in claim 22, wherein:  
  
the tissue-like organization of cells and macroscopic tissue like constructs are made without the requirement for scaffold or extraneous matrix or complex bioreactor for tissue generation to produce three-dimensional tissue-like constructs for implantation in a human or mammalian body as therapy for diseased or damaged conditions;

formation of the tissue-like constructs is free of the requirement for a pre-shaped well having a surface detrimental for cell attachment; and



the tissue-like organization of cells and macroscopic tissue-like constructs are made free of the requirement for any agents such as tissue-inducing chemicals, tissue-inducing growth factors, substratum with special properties, rotational culture.